MADROÑO, Vol. 65, No. 2, pp. 68–79, 2018

WATER AVAILABILITY INFLUENCES SPECIES DISTRIBUTIONS ON SERPENTINE SOILS

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ABSTRACT

Serpentine soils are highly variable environments, but how this variability contributes to maintaining distinct distributions of species at small scales is unclear. We studied parapatric populations of *Layia jonesii* A. Gray, a rare serpentine endemic, and *L. platyglossa* (Fisch. & C. A. Mey) A. Gray, a wide-spread species, to understand how edaphic variability and competitive interactions maintain co-occurring, somewhat interfertile, relatives within serpentine environments. We analyzed soil characteristics along a serpentine hillside where *L. jonesii* and *L. platyglossa* are restricted to the top and bottom of the hillside, respectively. We used reciprocal transplants to investigate fitness differences on the hillside and a competition experiment to determine if species interactions also restrict the distribution of *L. jonesii*. The soil analyses showed the top of the hill is drier than the bottom, yet both locations are chemically indistinguishable. *Layia platyglossa* is better adapted to the edaphic conditions at the bottom of the hillside and *L. jonesii* is sensitive to competition in the bottom region. Differences in soil water availability, and potentially its effect on the competitive environment, likely contributes to maintaining the distribution of these species. Ionic stress is often cited as a driver of species' distributions within serpentine environments, but our research suggests soil water availability can be an important factor influencing the distribution of interfertile species on serpentine at small scales. Annual variation in precipitation may drive local shifts in species distributions through alteration of the competitive environment.

Key Words: Competition, edaphic adaptation, *Layia*, local adaptation, plant distributions, plant soil relations, serpentine endemic, water availability.

Divergent natural selection across heterogeneous environments causes ecological differentiation between closely related species or ecotypes of the same species (Kawecki and Ebert 2004). This adaptation to specific environmental conditions contributes to maintaining spatial distributions of taxa by preventing the successful colonization of migrants from one habitat to another (Nosil et al. 2005; Sobel et al. 2010). Because ecological differentiation can control the distribution of plant species, it also directly contributes to reproductive isolation by limiting gene flow between interfertile taxa (McNeilly and Antonovics 1968; Kawecki and Ebert 2004). Investigating ecological differentiation between closely related species or ecotypes of the same species helps us understand what environmental factors maintain the distribution of plants across landscapes as well as how this can lead to speciation (Clausen et al. 1941; Angert and Schemske 2005; Lowry et al. 2008).

Divergent adaptation to different soil environments can strongly influence the distribution of plant species and is an important factor driving plant evolution (Kruckeberg 1954, 1986; Cornwell and Grubb 2003; Rajakaruna 2004; Baldwin 2005; Yost et al. 2012). Soils are highly heterogeneous environments with significant variability in chemical and physical characteristics, which can drive divergent selection among taxa (Ettema and Wardle 2002; Yost et al. 2012). In particular, soils that are considered harsh edaphic environments, either physically, chemically, or both, can be strong agents of natural selection (Rajakaruna 2004; Harrison and Rajakaruna 2011; Rajakaruna et al. 2014). Studies of plants growing on harsh soils derived from serpentine (Kruckeberg 1951; Main 1974; Brady et al. 2005; Wright et al. 2006), gypsum (Meyer 1986; Palacio et al. 2007; Moore et al. 2014; Escudero et al. 2015) and mine tailings (Antonovics 1968, 2006; McNeilly and Antonovics 1968) show local adaptation compared to neighboring soil types. Heterogeneity within a single soil type at small spatial scales can also provide sufficient variability for divergent selection (Lechowicz and Bell 1991; Yost et al. 2012; Chen and Schemske 2015).

Plants adapted to soils derived from ultramafic parent material (high in magnesium and iron, like serpentine) are often studied as a model system to understand how divergent environmental conditions can lead to differential adaptation, reproductive isolation, and speciation (Brady et al. 2005; Kay et al. 2011; Anacker 2014). Serpentine soils are harsh edaphic environments characterized by large magnesium to calcium ratios, elevated levels of toxic heavy metals, and deficient levels of plant essential macronutrients like phosphorus and potassium. Serpentine also weathers quickly causing soils to be highly erodible and dry (Alexander et al. 2007). These harsh chemical and physical conditions are hypothesized to drive divergent selection, and therefore differential adaptation, of serpentine vs. non-serpentine taxa (Rajakaruna 2003, 2004; Brady et al. 2005; Kay et al. 2011; Wright and Stanton 2011).

Traditionally, studies of ecological differentiation on serpentine soils involve comparing species or ecotypes growing in discrete soil types (i.e., serpentine vs. non-serpentine soils), but serpentine environments are highly heterogeneous, ranging from shallow rock outcrops to deep grassland soils. This variability has the potential to drive divergent selection, and therefore divergent adaptation, even at small spatial scales *within* serpentine environments (Gram et al. 2004; Harrison et al. 2006; Alexander et al. 2007). Divergent adaptation within serpentine environments can lead to ecological differentiation among taxa, which can reinforce reproductive isolation and drive speciation, as well as foster species co-existence through niche partitioning (Silvertown 2004). For example, Yost et al. (2012) found that differential adaptation to edaphic variation across a serpentine hillside maintains distinct distributions and reproductive isolation between two closely related species in the genus *Lasthenia* (Asteraceae) Cass. Observing and investigating the distribution of close relatives within serpentine environments can help us understand which factors allow relatives to co-exist in close proximity, and yet studies investigating such co-occurrences are scarce. Serpentine soils also support high concentrations of rare, endemic species (Safford et al. 2005; Harrison and Rajakaruna 2011; Anacker 2014), therefore understanding how environmental heterogeneity influences distribution of plant species can provide insight into why these areas are biologically diverse.

Some serpentine endemic species face a trade-off between serpentine tolerance and competitive ability, and it has been hypothesized that the suboptimal conditions found in serpentine environments serve as a refuge from competition (Kruckeberg 1954; Grime 1977; Gram et al. 2004; Brady et al. 2005; Kazakou et al. 2008; Harrison and Rajakaruna 2011). Grasslands on serpentine soils are less productive, and therefore provide a less competitive environment, than most non-serpentine soils (Moore and Elmendorf 2011). Although there is evidence that the distribution of serpentine endemics may be controlled by competition at a broad scale, it is unclear how competitive interactions influence the distribution of serpentine endemics within serpentine environments at a fine scale.

The genus *Layia* (Asteraceae) Hook. & Arn. ex DC. consists of rare edaphic specialists, as well as more widespread edaphic generalists, and therefore provides a good system to investigate how ecological differentiation and adaptation to specific edaphic environments maintains species' distributions and contributes to speciation (Clausen 1951; Baldwin 2005, 2006). In California, rare edaphic specialists like *L. jonesii* A. Gray and *L. discoidea* D.D. Keck (both restricted to serpentine soils) and *L. munzii* D.D. Keck (restricted to alkali soils) have extremely limited ranges. The genus also consists of widespread species, including *L. platyglossa* (Fisch. & C.A. Mey) A. Gray, which occurs through extensive portions of central and southern California in a wide variety of habitats and soils, and *L. glandulosa* (Hook.) Hook. & Arn., which occurs throughout the western United States and into Mexico in mostly sandy soils (Baldwin et al. 2012). Investigating edaphic factors that contribute to the wide spectrum of spatial distributions within this genus will help us assess the importance of these edaphic factors to species co-existence as well as speciation.

Here, we focused our investigation on *L. jonesii*, a rare serpentine endemic restricted to only a few populations in San Luis Obispo County, CA and *L. platyglossa*, a more common and widespread species. The widespread *L. platyglossa* is sister to a small clade containing *L. jonesii*, *L. munzii*, and *L. leucopappa*. In other words, while *L. platyglossa* is a close relative of *L. jonesii*, they are not sister species, but they do retain modest interfertility (Clausen 1951; Baldwin 2003). In San Luis Obispo, these two species often co-occur within a few meters of each other (parapatrically) on serpentine derived grassland hillsides (CCH 2017). At sites where the two species are known to co-occur, each species has a distinct, non-overlapping distribution. *Layia jonesii* occurs mainly on the top of the hillsides, where the mostly native vegetation is sparse and patchily distributed and where serpentine rock outcrops are exposed. *Layia platyglossa* occurs mainly on the bottom of the hillside where the vegetation is denser and composed of annual and perennial forbs as well as native and non-native grasses. Although the two species have distinct distributions at sites where they co-occur, there is little visible evidence of a sharp division of the two regions into environmentally distinct habitats. These species provide an opportunity to investigate how heterogeneity within a serpentine edaphic environment can contribute to the maintenance of distinct species distributions, and therefore can help broaden our understanding of how this heterogeneity can contribute to speciation and high levels of endemism in serpentine environments.

Soil chemical and physical properties, as well as the competitive environment, vary across small spatial scales within serpentine habitats and could influence the distribution of species (Alexander et al. 2007; Moore and Elmendorf 2011). Reciprocal transplants are a powerful way to test for fitness differences between regions within serpentine, and, when combined with a thorough analysis of the soil environment, transplants can help us understand which edaphic factors control the distribution of species due to differential adaptation (Nagy and Rice 1997; Angert and Schemske 2005; Sambatti and Rice 2006; Wright et al. 2006; Yost et al. 2012; Chen and Schemske 2015). Here, we use reciprocal transplants combined with a competition experiment (neighbor removal) and a soil analysis to address the potential mechanisms that maintain the fine-scale spatial separation between *L. jonesii* and *L. platyglossa*. We tested four hypotheses to understand how fitness of the two species and soil characteristics differ along the hillside. We addressed the following hypotheses about the fitness of the two species across the hillside: (1) each species will have a higher fitness within its natural distribution than outside its natural distribution, and (2) the removal of neighbors will increase the fitness of *L. jonesii* outside its natural distribution at the bottom of the hill. We addressed the following hypotheses regarding the edaphic factors that could contribute to maintaining the unique distribution of *Layia:* (3) there are small-scale chemical differences between the soils in the two regions, and (4) there are small-scale physical differences between the soils in the two regions. It is important to note that these variables likely do not independently affect the distribution; rather, the distribution of *Layia* may be controlled by a combination of these factors.

METHODS

Study System

The genus *Layia* consists of 14 spring-flowering annual species native to California. Most species are self-incompatible, including *L. jonesii* and *L. platyglossa* (Clausen 1951). *Layia jonesii* and *L. platyglossa* are reliably distinguished morphologically by pappus shape. *Layia jonesii* has a pappus of short scales, whereas *L. platyglossa* has pappus of long bristles. *Layia jonesii* is endemic to San Luis Obispo County, CA on serpentine derived soils (Safford et al. 2005). The distribution of *L. jonesii* consists of only about 15 small, scattered locations from the city of San Luis Obispo north to Cayucos (CCH 2017). In contrast, *L. platyglossa* is a more widespread species ranging from Baja California to the northern coast ranges of California, and occurs on a range of soils, including serpentine (Clausen 1951; Baldwin et al. 2012). Herbarium records document the two species growing parapatrically (as adjacent populations) at multiple populations within San Luis Obispo County (CCH 2017).

At one of these locations, the two species occur within meters of each other on a serpentine-derived hillside in Reservoir Canyon Open Space (RC), San Luis Obispo County, CA (35.28490, –120.61830). This distribution is characteristic where *L. jonesii* and *L. platyglossa* co-occur. RC is part of a series of serpentine ridges that run parallel to the Santa Lucia Range, and are part of the Franciscan Complex, a coastal geologic formation consisting of mainly metamorphic substrates, including serpentine.

Reciprocal Transplant and Competition Experiment

To determine if either species is adapted to the specific edaphic environment on the region of the hillside where it occurs, we performed a reciprocal transplant between *L. jonesii* and *L. platyglossa* at RC. We established four reciprocal transplant plots (0.5m x 0.5m) on a serpentine hillside where *L. jonesii* and *L. platyglossa* co-occur. Two plots were placed at the top of the hillside in the region where *L. jonesii* occurs, and two plots were placed at the bottom of the hillside in region where *L. platyglossa* occurs. The plots at the top of the hill were approximately 40m upslope from the plots at the bottom of the hill. In each of the four plots, we planted two single-seeded fruits (achenes or cypselae, herein called seeds) from 16 maternal families of each species. Seeds were collected from RC during the previous season. In total, we planted 32 seeds (16 families) of *L. jonesii* and 32 seeds of *L. platyglossa* (16 families) for a total of 64 seeds per plot. We glued each seed to a color-coded toothpick to assist with tracking each plant through its life cycle. Each seed was randomly assigned a planting position within the plots. We planted the seeds in early November 2013.

To determine whether interspecific competitive interactions restrict the range of *L. jonesii* to the top of the hill, we conducted a competition experiment known as a neighbor removal experiment. In both of the transplant plots at the bottom of the hill, we established two smaller sub-plots (weeded plots) adjacent to the reciprocal transplant plots in which we planted two additional seeds from each of the same 16 maternal families of *L. jonesii*. On a weekly basis, we weeded out all plants except for the planted *L. jonesii* seedlings.

To document fitness across multiple life history transitions, we recorded germination, survival, and flowering time in the plots on a weekly basis until the end of the growing season. We quantified fitness using three measures: proportion of seeds germinated, proportion of geminated seeds that surviving to flowering, and viable seed set (total reproductive fitness). We quantified seed set by counting the number of viable seeds produced by each seed planted in both the reciprocal transplant and competition experiment plots. Seeds that failed to germinate were considered to have a fitness of zero. Dark-colored, swollen achenes were assumed to contain a viable seed. We also measured peak flowering time, which was considered to be when the highest proportion of open heads were present within the plots. An open head was defined as having at least one open disk floret.

We analyzed germination success and survival to flowering success in the plots using generalized linear models (GLM), and we analyzed viable seed set in the reciprocal transplant plots using a generalized regression with a zero-inflated Poisson distribution to account for many zero values within our dataset. We analyzed viable seed set in the competition plots using GLM. All analyses were performed with JMP (JMP 12.1.0, SAS Institute Inc., Cary, NC). To understand the effect of plot and maternal family on these analyses, we first ran our models by including

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Justen Whittall, *Madroño* Editor, with Arjun (left) and Ayana (right)

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- **Caitlin Sullivan, Holy Cross School, Santa Cruz, CA:** Effects of X-Ray Radiation on Plants (1st place Middle School division)
- **Ashlyn McDaniel, Baymonte Christian School, Scotts Valley, CA:** Vinegar Stops Mold on Strawberries (2nd place Middle School division)

Justen Whittall with (starting from left) Ashlyn, Caitlin and Emma

Plot (nested within region) and Family (nested within species) as fixed effects (full model). When Plot or Family fixed effects were not significant ($P < 0.05$), we excluded them from our analysis and ran a reduced model. We report AICc values for full and reduced models, and our figures are reported using the model with the lowest AICc.

Soil Sampling

After the completion of the transplant experiment, we sampled soil from the four reciprocal transplant plots to characterize the soil chemistry at RC. We made three, 1-liter collections of soil from the top 15cm in each plot. The soil was tested at A&L Western Laboratories for organic matter percent, estimated nitrogen release (ENR, lbs/acre), phosphorus (Weak Bray and Olson Method, ppm), potassium (ppm), magnesium (ppm), calcium (ppm), sodium (ppm), soil pH, hydrogen (meq/100g), cation exchange capacity (CEC, meq/100g), and sulfur (ppm). We performed a principal component analysis with these soil characteristics using JMP.

To determine the physical characteristics of the soils at RC, we conducted one soil profile (pedon) description in the *L. jonesii* region (top) and one in the *L. platyglossa* region (bottom) of the hillside. Within each region, we classified the soil directly adjacent to a reciprocal transplant plot by digging a soil pit to the bedrock level and exposing a vertical face of the soil profile to examine the horizons (layers), as is best practice in soil science (Soil Science Division Staff 1993). We described the depth, rock fragment content (using a sieve), texture, and structure for each horizon in both soil profiles. Each pedon description is representative of its respective region (Soil Science Division Staff 1993).

We also calculated the available water holding capacity (AWHC) for each soil profile using standard calculations based on texture, soil depth, and the proportion of rock fragments (Soil Science Division Staff 1993; Schoeneberger et al. 2012). Soils store water in soil pores, and some of that water is available to plants to absorb and use. The available water holding capacity (AWHC) is the amount of water (in cm) that is potentially available to plants. The AWHC is calculated for each soil horizon using the following equation:

$$\left[\textit{textural class AWHC}\left(\frac{\textit{cm AWHC}}{\textit{cm soil}}\right)\right] \times [\textit{depth of horizon}\,(\textit{cm soil})] \times [1 - \textit{prop.rock fragments}]$$

The values for each horizon are added to calculate the AWHC of the entire soil profile. Using USDA-NRCS convention, we did not include weathered bedrock horizons (Cr) in our AWHC calculations because these horizons consist of mainly weathered rocks that do not possess water storage potential (Soil Science Division Staff 1993). We also did not include the influence of rock fragments on soil horizons that contained <5% rock fragments by volume (Soil Science Division Staff 1993). All horizons in our two pedons had a clay texture. The AWHC for clay soils is 0.15 cm AWHC/cm soil (Kramer 1983).

TABLE 1. Principal component analysis of chemical soil characteristics between the *Layia jonesii* region at the top and the *L. platyglossa* region at the bottom of the hill on a serpentine hillside in Reservoir Canyon. Bold values represent loading scores above 0.5. CEC is the cation exchange capacity and ENR is the estimated nitrogen release.

Soil character	PC1 (35.8%)	PC2 (31.5%)
Organic matter (%)	−0.1315	**0.9322**
ENR (lbs/A)	−0.1299	**0.9166**
Phosphorus – weak Bray (ppm)	**0.8316**	−0.2535
Phosphorus – Olsen method (ppm)	**0.7637**	−0.2012
K (ppm)	**0.7925**	−0.1463
Mg (ppm)	0.0826	**0.9351**
Ca (ppm)	**0.8522**	0.2401
Na (ppm)	−0.0032	**0.6035**
Soil pH	**−0.8690**	−0.3383
H (meq/100g)	−8.29E-17	2.17E-16
CEC (meq/100g)	0.3116	**0.9106**
Sulfur (ppm)	**0.6659**	0.0555
Ca:Mg (%)	**0.8242**	-0.1388

RESULTS

Soil Characteristics

To characterize the chemical edaphic environment on the serpentine hillside, we conducted a principal component analysis of 13 chemical soil characteristics in both the *L. jonesii* (top of hillside) and the *L. platyglossa* (bottom of hillside) regions. The first two principal components describe 67.3% of the variation in soil characteristics between the two regions in RC (Table 1, Fig. 1). The analysis shows no chemical differentiation between the soils in the two regions. Soils from both regions have low levels of essential nutrients like phosphorus, potassium, and calcium along with high levels of magnesium (loading scores above 0.5), a pattern characteristic of serpentine soils (Alexander et al. 2007).

To characterize the physical edaphic environment on the serpentine hillside, we conducted a soil profile (pedon) characterization in both the *L. jonesii* and *L. platyglossa* regions. Both soil profiles show evidence of soil development by the presence of accumulated clay in the subsurface horizon (B_t). The presence of this horizon in profiles indicates that both soils are stable (Buol et al. 2003). The soils in both regions are of similar depth to weathered bedrock (top 20cm, bottom 25cm; Table 2), however, the soil at the top of the hill in the *L. jonesii* region contains a higher percentage of rock fragments in all solum horizons

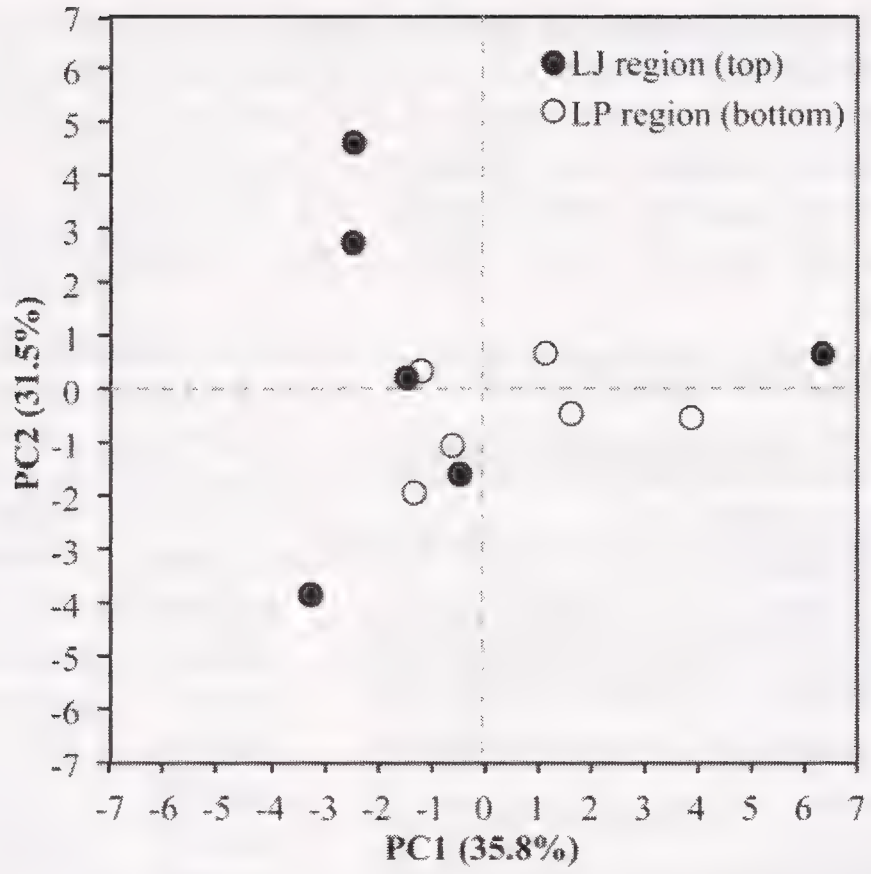

FIG. 1. Principal component analysis of the 13 chemical soil characteristics. The first two principal components (PCs) describe 67% of the variation in soil characteristics between the two regions at RC. The points in the plot above represent individual soil samples from the *L. jonesii* region at the top of the hill (black circles) and the *L. platyglossa* region at the bottom of the hill (white circles). See Table 1 for loading scores.

(horizons above the bedrock, C_r) compared to the soil in the *L. platyglossa* region.

The presence of a high percentage of rock fragments in the solum reduces the available water holding capacity (AWHC) of the soil. The water that is available to plants is held within the pores of the soil matrix, therefore, any factor that reduces the total volume of the soil matrix also reduces the available water holding capacity. Both rock fragments and soil depth can influence the total volume of the soil matrix (Hillel 2004). For example, shallow soils have less soil volume, therefore less available water holding capacity than deep soils. Similarly, rock fragments reduce the total volume of the soil matrix, therefore, rocky soils have less available water holding capacity than soils without rocks. The available water holding capacity of the soil in the *L. jonesii* (LJ) region is about half of the water holding capacity of the soil in the *L. platyglossa* (LP) region (soil in LJ region 1.29 cm of water; soil in LP region 3.75 cm of water; Table 2).

Reciprocal Transplant

We sought to determine if *L. jonesii* or *L. platyglossa* are differentially adapted to specific edaphic regions on the hillside at RC using a reciprocal transplant. To quantify plant fitness during the early portion of the life cycle, we tracked the germination success (LJ at top n = 62, at bottom n = 63; LP at top n = 63, at bottom n = 63) and survival to flowering success (LJ at top n = 21, at bottom n = 25; LP at top n = 20, at bottom n = 15) in the plots. The proportion germinating was higher in *L. jonesii* than *L. platyglossa* ($\chi^2 = 5.845$, P = 0.0156; Table 3; Fig. 2A). The proportion surviving to flowering of *L. jonesii* and *L. platyglossa* was not significantly different in the plots in both the top and bottom regions (Table 3; Fig. 2B). We also tracked the overall survival of germinated seedlings in the plots over time throughout the duration of the experiment. In the *L. jonesii* region (top of hillside), we observed that *L. platyglossa* had higher mortality than *L. jonesii* during the beginning of the experiment. During early April, the proportion of *L. platyglossa* seedlings surviving in the *L. jonesii* region dropped to 53% while the proportion of *L. jonesii* seedlings surviving in the same region remained at 100% (Fig. 3A, B). The overall survival of both species in the *L. platyglossa* region (bottom of hillside) was not different.

We used viable seed set of all individual seeds planted to determine the reproductive fitness of *L. jonesii* and *L. platyglossa* in the two regions on the hillside in RC (LJ at top n = 62, at bottom n = 63; LP at top n = 63, at bottom n = 63). Results from the reciprocal transplants showed the reproductive fitness of both *L. jonesii* and *L. platyglossa* was higher at the bottom of the hill in the *L. platyglossa* region than at the top of the hillside in the *L. jonesii* region (LJ at top 2.42 ± 3.84 mean viable seeds per individual, at bottom 15.28 ± 5.21 mean viable seeds per individual; LP at top 0.86 ± 3.04 mean viable seeds per individual, at bottom 5.26 ± 5.23 mean viable seeds per individual; $\chi^2 = 12.332$, P = 0.0004; Table 3; Fig. 2C). *Layia jonesii* showed a somewhat higher fitness in both regions on the hillside compared to *L. platyglossa* ($\chi^2 = 8.759$, P = 0.0031; Table 3; Fig. 2C).

TABLE 2. Soil profile descriptions for soil pits at the *L. jonesii* (LJ) region at the top and the *L. platyglossa* (LP) region at the bottom of the hill. Available water holding capacity (AWHC) in cm of water for the entire soil profile is provided for each region.

Region	AWHC (cm)	Horizon	Depth (cm)	Rock fragments	Texture	Structure
LJ	1.29	A	0–1.5	<5%	Clay	Granular
		Bt	1.5–6	50%	Clay	Blocky
		Bt/C	6–20	65%	Clay	Blocky
		Cr	20+	-	-	-
LP	3.75	A	0–5	<5%	Clay	Granular
		Bt	5–25	<5%	Clay	Blocky
		Cr	25+	-	-	-

TABLE 3. A summary of the generalized linear models (germination and survival to flowering analyses) and generalized regression (viable seed set) for the fixed effects of region (top or bottom of hill), species, region x species, plot (nested within region), and family (nested within species) on the germination, survival to flowering, and viable seed set of *L. jonesii* and *L. platyglossa* in the reciprocal transplant plots. The full model includes all fixed effects. The reduced model excludes plot or family when not significant ($\alpha = 0.05$) in the full model. The chi-squared value for the viable seed set analysis is the Wald chi-squared value. The AICc values are presented for all models. Values in bold indicate significant effects.

		Germination		Survival to flowering				Viable seed set			
		Full model AICc = 334.11		Full model AICc = 182.17		Reduced model AICc = 112.64		Full model AICc = 453.03		Reduced model AICc = 450.91	
Fixed effect	df	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Region	1	0.048	0.8259	0.723	0.3951	1.489	0.2223	10.440	**0.0012**	12.332	**0.0076**
Species	1	5.845	**0.0156**	1.599	0.2059	1.467	0.2254	16.593	**<0.0001**	8.759	**0.0200**
Region x Species	1	1.508	0.2195	2.540	0.1110	1.193	0.2746	0.085	0.7704	0.062	0.5524
Plot [Region]	2	54.800	**0.0037**	29.274	0.2988	-	-	2.693	0.2601	-	-
Family [Species]	30	9.156	**0.0103**	2.177	0.3368	-	-	84.217	**<0.0001**	51.43	**0.0088**

We also tracked flowering times of both species in the reciprocal transplant plots. In both regions, peak flowering time of *L. jonesii* occurred about a week earlier than *L. platyglossa*. Peak flowering time of both species occurred about a week earlier at the top of the hillside in the *L. jonesii* region than at the bottom of the hillside in the *L. platyglossa* region (Fig. 3A, B). Although *peak* flowering time was different between species and regions, a portion of each species' flowering cycles overlapped. For example, during peak flowering time of *L. jonesii* in the region at the top of the hill, individuals of *L. platyglossa* were blooming but the individuals in the plot had not yet reached peak flowering.

Competition Experiment

We sought to determine if the distribution of the rare serpentine endemic *L. jonesii* is constrained to the region at the top of the hillside because of interspecific competitive interactions at the bottom of the hillside. Results from the competition experiment at the bottom of the hillside showed that germination (LJ without weeding n = 63, with weeding n = 64) and survival to flowering (LJ without weeding n = 25, with weeding n = 26) of *L. jonesii* in plots at the bottom of the hillside were not significantly different for weeded and un-weeded plots ($\chi^2 = 0.003$, $P = 0.95$; and $\chi^2 = 0.05$, $P = 0.81$, respectively; Table 3; Fig. 2D, E). The *L. jonesii* individuals had a higher average reproductive fitness when neighbors were removed in the weeded portion of the plots than when neighbors were not removed (LJ without weeding 15.28 ± 17.25 mean viable seeds per individual; LJ with weeding 94.35 ± 53.9 mean viable seeds per individual; $F = 4.49$, $P = 0.036$; Fig. 2F). The increase in reproductive fitness of LJ in the weeded portion of the plot is likely due to decreased competition for resources such as water, nutrients, or light rather than increased pollinator efficiency due to increased visibility of floral heads to pollinators. Plants in the weeded portion of the plots were more robust and produced more floral heads. Removal of competitors was accompanied by an increased in the average number of floral heads per individual from 2.35 to 8.71.

DISCUSSION

Differential adaptation and edaphic specialization can control the distribution of plant species at a local scale (Lechowicz and Bell 1991; Yost et al. 2012). Heterogeneity *within* serpentine environments can potentially drive divergent selection and allow for the co-existence of close relatives (Kay et al. 2011; Yost et al. 2012). Studies investigating variability within serpentine environments can help us to further understand which edaphic factors influence distributions and co-existence of species at small scales. At RC, the distinct distribution of two species in the genus *Layia* on a serpentine hillside provided an opportunity to study how heterogeneity within

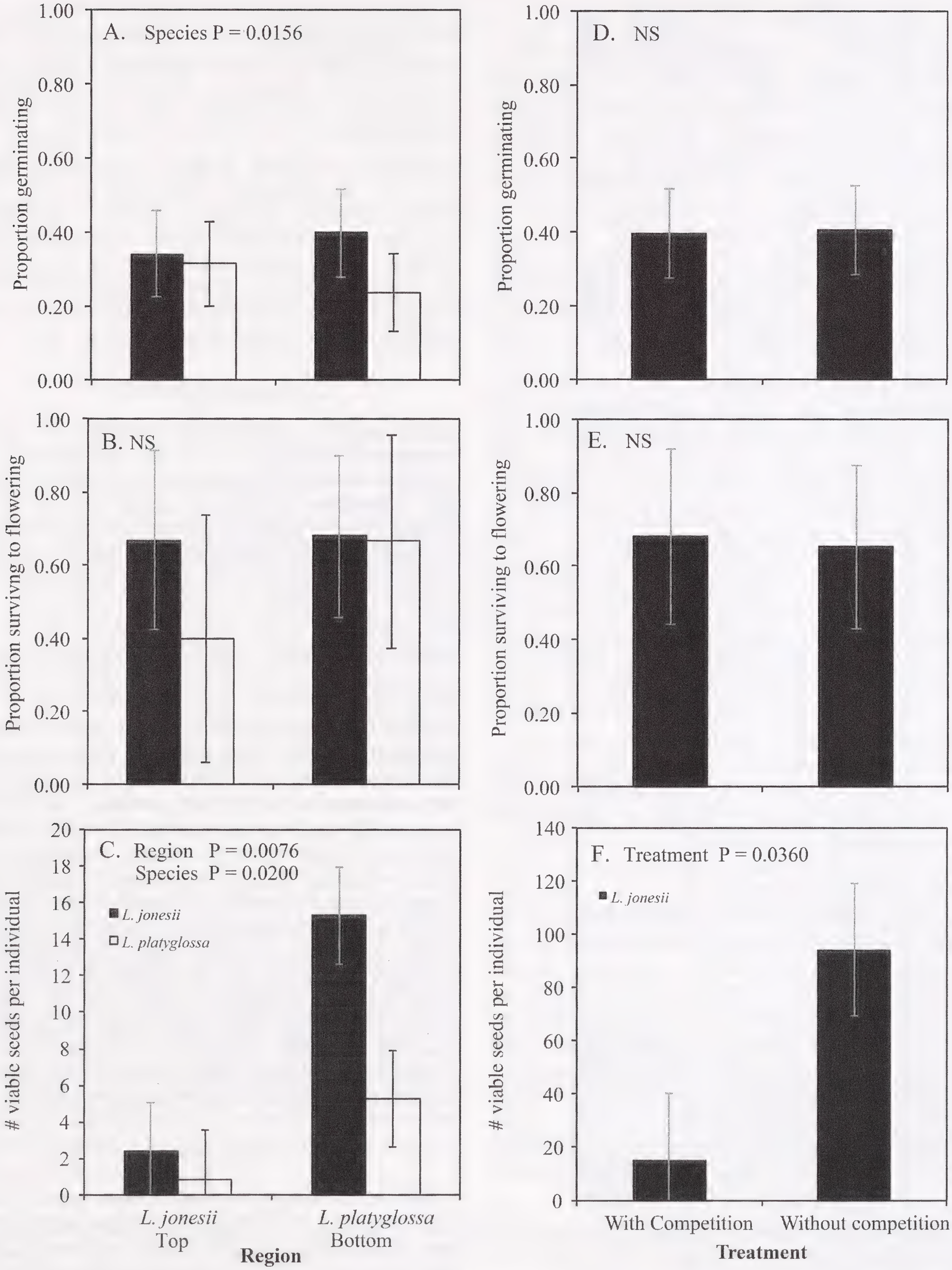

FIG. 2. Fitness parameters measured for *L. jonesii* (black bars) and *L. platyglossa* (white bars) during the reciprocal transplant and competition experiments. The left column shows the results from the reciprocal transplant experiment: (A) germination, (B) survival to flowering of germinated individuals, and (C) number of viable seeds produced per individual seed planted. The right column shows the results from the competition experiment: (D) germination, (E) survival to flowering of germinated individuals, and (F) number of viable seeds produced per individual seed planted. Bars in graphs (A), (B), (D), and (E) represent proportions. Bars in graphs (C) and (F) represent the average number of viable seeds produced by *L. jonesii* and *L. platyglossa* within each region or treatment. Only significant effects ($P < 0.05$) from the analyses are listed in each graph. Non-significance is denoted as NS. See Table 3 for a summary of the analyses. Error bars in (A), (B), (D), and (E) represent a 95% confidence interval. Error bars in (C) and (F) represent ± 1 SE.

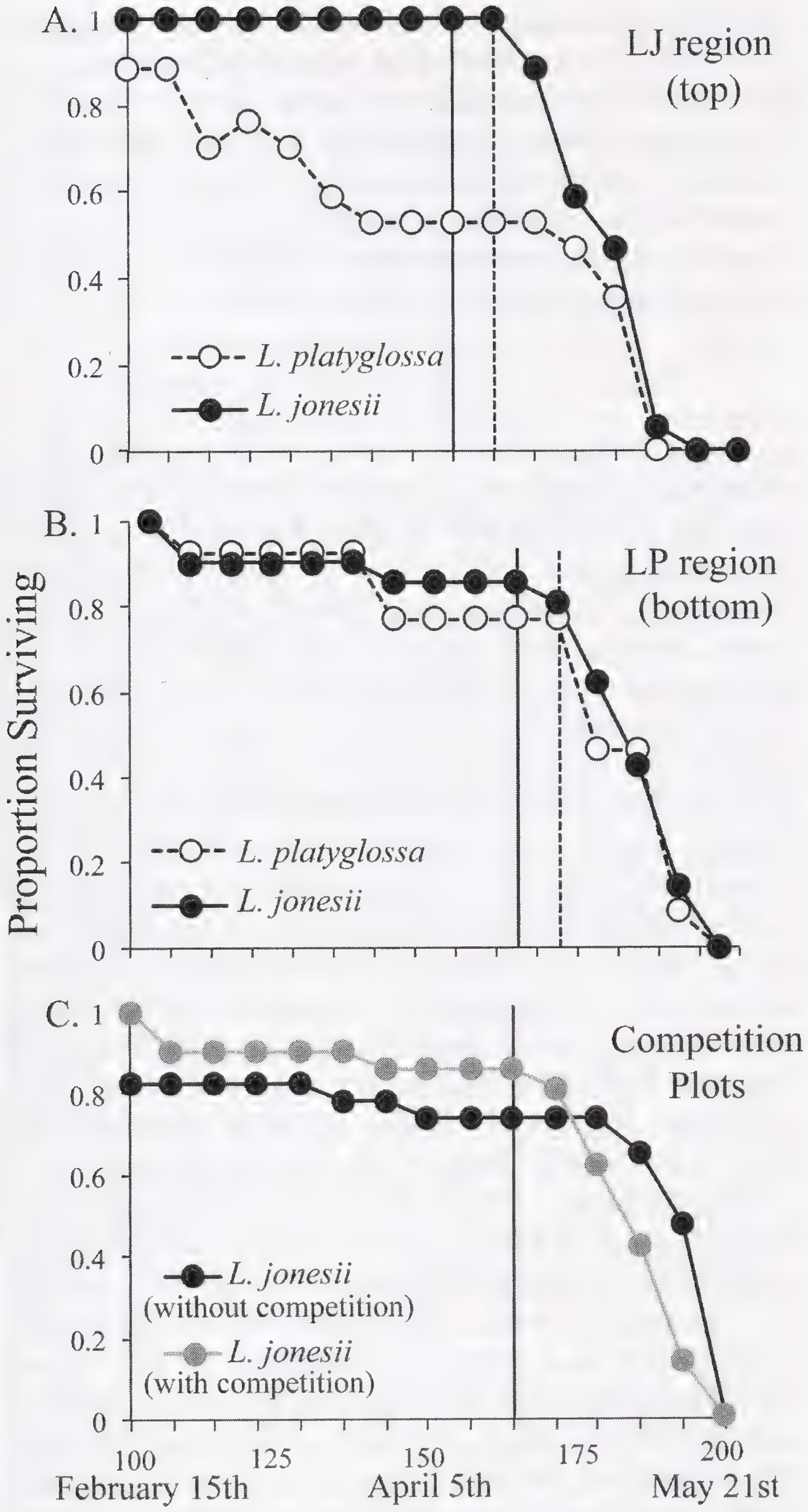

FIG. 3. Overall survival of germinated seedlings over time since planting of seeds in the transplant plots in the (A) *L. jonesii* region at the top of the hill, (B) *L. platyglossa* region at the bottom of the hill, and (C) competition plots (with and without weeding) in the *L. platyglossa* region at the bottom of the hill. The vertical solid line represents peak flowering time for *L. jonesii* and the vertical dashed line represents peak flowering time for *L. platyglossa* within the plots. In the competition plots, *L. jonesii* in plots with and without weeding had the same peak flowering time and it is denoted by a single solid vertical line.

serpentine environments can contribute to maintaining distinct distributions and to understand how these factors may contribute to high serpentine diversity. We found that soil water availability caused by differences in physical soil properties likely controls the distinct distribution of the two species.

Soil Environment

Because serpentine soils are chemically extreme, most studies of plant distributions within serpentine environments focus on chemical properties of the soils and largely overlook important physical properties, such as soil depth, soil texture, and rock fragment content. These properties can have an important influence on water and plant relationships (Kramer 1983; Hillel 2004), and may be an important factor influencing plant distributions. Our study is one of the first to compare both chemical and physical properties of serpentine soils between two regions to understand how edaphic factors influence species' distributions.

Soils in the *L. jonesii* region near the top of the hill and the *L. platyglossa* region near the bottom of the hill were chemically indistinguishable, therefore, soil chemistry is not a factor contributing to the distinct distribution of *Layia* in RC. Although our study unexpectedly found the soils to be chemically similar, other studies have found the soil chemical environment is an important factor contributing to the coexistence of species within serpentine environments (Rajakaruna and Bohm 1999; Rajakaruna et al. 2003b). Yost et al. (2012) found that ionic differences along an edaphic gradient were an important factor driving differential fitness between closely related species of *Lasthenia*.

Although the soils are chemically similar, they are physically distinct. The soils near the top of the hill in the *L. jonesii* region contain a higher percentage of rock fragments in each horizon than soils at the bottom of the hill. Because of the high rock fragment content, soils at the top of the hill can hold about half as much water as the soils at the bottom of the hill. This means there is less soil water available to plants in the *L. jonesii* region. Because of its decreased water holding capacity, the *L. jonesii* region is expected to become drier earlier in the spring than soils in the *L. platyglossa* region. This phenomenon can easily be observed at RC and other similar sites, where the annual grasses and forbs near rocky outcrop regions flower and senesce earlier than plants in more densely vegetated grasslands regions. This accelerated phenology is likely a way for these individuals to escape impending seasonal drought (Heschel and Riginos 2005; Wu et al. 2010).

Spatial differences in soil water availability have been shown to be an important environmental mechanism influencing the distribution of species at both local and broad scales. Engelbrecht et al. (2007) showed drought sensitivity of various trees in tropical forest ecosystems controls the spatial distributions of species across a rainfall gradient in Panama. Differences in drought sensitivity can also contribute to maintaining distributions between closely related taxa at smaller spatial scales as well. Chen and Schemske (2015) found that variation in soil moisture is an important factor influencing the distribution of two closely related species in the tropical ginger genus *Costus*. Within serpentine environments, soil water availability has also been linked to divergent adaptation. Temporal differences in water availability between a seep and riparian

TABLE 4. A summary of the general linearized models (GLM) for the treatment (non-weeded vs. weeded plots), plot (nested within treatment), and family on the germination, survival to flowering, and viable seed set of *L. jonesii* in the competition plots. The full model includes all fixed effects. The reduced model excludes plot and family when not significant ($\alpha = 0.05$) in the full model. The AICc values are presented for all models. Values in bold indicate significant effects.

		Germination				Survival to flowering		Viable seed set			
		Full model AICc = 192.84		Reduced model AICc = 175.19		Full model AICc = 98.62		Full model AICc = 864.58		Reduced model AICc = 846.97	
Fixed effects	df	χ^2	P	χ^2	P	χ^2	P	F	P	F	P
Treatment	1	0.003	0.9570	0.011	0.9137	0.056	0.8124	4.188	**0.0431**	4.493	**0.0360**
Plot [Treatment]	2	1.386	0.5000	-	-	24.476	**0.0401**	0.556	0.5747	-	-
Family	15	22.324	0.0996	-	-	3.425	0.1804	1.541	0.1033	-	-

environment help to maintain divergent populations of a serpentine endemic sunflower (Sambatti and Rice 2006). Differential responses of two species of *Lasthenia* to water stress suggest water availability could be an important factor influencing the distinct distributions, and therefore reproductive isolation, of closely related species within serpentine environments (Rajakaruna et al. 2003a; Yost et al. 2012).

Selective Pressures within Serpentine

Reproductive fitness data and survival data suggest *L. platyglossa* is better adapted to the region near the bottom of the hillside. Reproductive fitness data (viable seed set) from the reciprocal transplant showed *L. platyglossa* was less fit in the rocky *L. jonesii* region near the top of the hillside than in its own region near the bottom of the hillside (Fig. 2C). Overall survival data also showed that *L. platyglossa* was less fit near the top of the hillside than at the bottom. The dry conditions found near the top of the hillside likely explain the fitness differences we detected between the two regions. Because the top of the hillside becomes drier earlier in the season, *L. platyglossa* in the transplant plots may not have been able to allocate sufficient resources to survival and reproduction under drought stress conditions, and this may limit the potential for the species to expand into the *L. jonesii* region. Future work in the greenhouse could directly test this hypothesis.

Unexpectedly, the reciprocal transplant did not support the idea that *L. jonesii* is best adapted to the edaphic conditions present near the top of the hillside in its own region. Although we observed the distribution of *L. jonesii* constrained to the top of the hill during 2013, the year before our transplant, our results indicate *L. jonesii* had a higher fitness near the bottom of the hill in the *L. platyglossa* region. These results could be related to the extreme drought that coincided with our reciprocal transplant in spring 2014, which was California's worst drought on record (Swain et al. 2014). During spring 2014, we observed that the natural distribution of *L. jonesii* expanded out from the rock outcrop region at the top and into the grassland region toward the bottom of the hillside. Extreme drought likely reduced the survival of many annual species in the grassland region, especially non-native grasses, which have been found to be especially sensitive to changes in precipitation on serpentine soils (Hobbs et al. 2007). Therefore, competition may have been lower in the grassland region during the drought than in previous years. Results from our competition experiment showed that *L. jonesii* is sensitive to competition from surrounding species. Reduced competition caused by drought may have allowed the *L. jonesii* individuals in our reciprocal transplant plots to take advantage of the more conducive growing environment, with higher water availability, found in soils in the grassland region near the bottom of the hill. The hypothesis that increased rainfall can lead to increased productivity, and therefore increased competition on serpentine soils, is supported by other studies. Harrison et al. (2006) found that increased rainfall and the corresponding increased productivity was negatively correlated with serpentine endemic richness on serpentine rock outcrops in California. This result supports our hypothesis that increased fitness of *L. jonesii* in the grassland region could be due to reduced rainfall, and therefore reduced competition.

Reduced competitive ability of some serpentine endemics may be due to an evolutionary trade-off between serpentine tolerance and competitive ability (Kruckeberg 1954; Grime 1977; Kazakou et al. 2008; Kay et al. 2011). It has been hypothesized that serpentine soils serve as a refuge from competition for many serpentine endemics that may be poor competitors off serpentine (Kruckeberg 1954; Gram et al. 2004; Brady et al. 2005; Alexander et al. 2007). Our results suggest that competitive interactions could influence the spatial distribution of serpentine endemics at an even finer scale. The dry rock outcrop habitats patchily distributed on serpentine soils may serve as refugia from competition for endemic species within the serpentine environments (Gram et al. 2004). If non-endemic competitors are able to tolerate the chemical extremes of serpentine, they might not also be able to tolerate drought. Therefore, heterogeneity of water availability could contribute to maintaining biodiversity (Silvertown 2004).

Our anecdotal observation of *L. jonesii* expanding its local range during a drought year suggests there are very complex interactions between biotic and abiotic factors in this system. The distribution of *L. jonesii* and *L. platyglossa* may be dynamic from year to year, based on annual environmental conditions and interactions between species. This dynamic distribution pattern suggests natural selection varies from year to year based on climatic fluctuations. These inter-annual fluctuations may maintain the distribution of the two populations on serpentine-derived hillsides, where the expansion of the rare, and potentially more drought tolerant serpentine endemic *L. jonesii,* is favored during dry years, but expansion of the common *L. platyglossa* is favored during wet years. Our results are consistent with previous studies that suggest differences in drought tolerance can influence the co-existence and the distribution, and therefore reproductive isolation, of taxa or ecotypes within heterogeneous serpentine environments (Gardner and Macnair 2000; Hughes et al. 2001; Rajakaruna et al. 2003a; Sambatti and Rice 2006).

There are other examples of widespread members of the genus *Layia* co-occurring with rare congeners. For example, *L. platyglossa* co-occurs with the rare *L. munzii* D.D Keck on alkali soils in eastern San Luis Obispo County, California. These types of co-occurrences may be maintained by mechanisms similar to those reported here, such as differential adaptation to specific edaphic environments at a local scale. Investigating these kinds of co-occurrences can provide insight into how rare species are maintained near interfertile and widespread relatives.

Flowering Time Differences

We also found evidence that peak flowering time for *L. jonesii* occurs earlier than *L. platyglossa.* In all plots, peak flowering time for *L. jonesii* occured about a week earlier than *L. platyglossa.* Earlier flowering likely allows *L. jonesii* to complete its lifecycle and reproduce before the onset of the most intense period of seasonal drought negatively impacts the reproductive fitness of this species. We also observed that both species flowered earlier in the drier rock outcrop habitat than the grassland habitat. In this case, earlier flowering time of both species may be a plastic response to the dry conditions of the rock outcrop habitat. These patterns suggest that both genetic and environmental factors may control the flowering time of *L. jonesii* and *L. platyglossa.*

Drought can drive selection for earlier flowering times that shorten the life cycle and allow for reproduction before the onset of drought (Sherrard and Maherali 2006; Wu et al. 2010; Franks 2011). Species that are unable to complete their life cycle before the onset of seasonal drought or that cannot effectively allocate resources to reproduction under drought stress will likely have a lowered fitness on dry soils (Chaves et al. 2003; Wu et al. 2010). Because drought can be a driver of selection for earlier flowering times, especially on dry serpentine rock outcrops, the rare serpentine endemic *L. jonesii* may be under strong selection for earlier flowering time. This is consistent with other studies that found some serpentine ecotypes flower earlier than non-serpentine ecotypes (O'Dell and Rajakaruna 2011). Although earlier flowering is associated with some serpentine ecotypes and taxa, flowering time shifts are not always associated with serpentine tolerance (Schneider 2017).

CONCLUSION

Edaphic factors influence the distribution of plant species, even at small scales. We found evidence that the distinct distribution of *L. jonesii* and *L. platyglossa* on a serpentine-derived hillside during our study period is maintained by physical, not chemical, edaphic factors. *Layia platyglossa* likely does not expand into the rock outcrop habitat because it is too dry, and the distribution of *L. jonesii* generally may be constrained by competitive interactions from surrounding species. Ionic stress is often cited as a driver of species' distributions within serpentine environments (Rajakaruna and Bohm 1999; Rajakaruna et al. 2003b), but our research suggests soil water availability is also an important factor influencing the distribution of species. We also observed a local population expansion of *L. jonesii* during an extreme drought, suggesting that temporal selection due to soil water availability may control